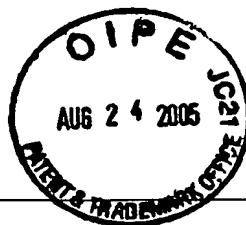


SUBSTITUTION SPECIFICATION
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- 1 -

TITLE OF THE INVENTION

A METHOD FOR ACQUIRING INFORMATION OF A BIOCHIP USING TIME
OF FLIGHT SECONDARY ION MASS SPECTROMETRY AND AN APPARATUS
5 FOR ACQUIRING INFORMATION FOR THE APPLICATION THEREOF

BACKGROUND OF THE INVENTION

Field of the Invention

[0001] The present invention relates to an imaging of
~~respective-a~~ matrix disposed on a surface of a biochip,
10 ~~that which~~ includes a substrate and a plurality of
biological ~~related-materials~~ disposed on a surface of the
substrate in a matrix form, and also relates to an analysis
of the components of ~~respective-the~~ matrix.

Description of the Related Art

15 [0002] A biochip, such as a DNA chip, protein chip and so
on, which includes a substrate and various ~~probe-molecular~~
~~probes~~ disposed on a surface of the substrate in a matrix
form, has been employed for the purposes of analyzing a
genome or analyzing a generation of a gene. Further, it is
20 expected that the result of the analysis ~~by-using-the~~
~~employing~~ biochips provides a critical index for diagnosis
of cancers, genetic diseases, life style-related diseases,
~~infectious~~ diseases and the like, prediction for

prognostics, or a decision of on treatment policy and so on.

[0003] Several methods for preparing biochips are known.

~~On describing the methods for preparing a DNA chip as~~

5 ~~examples, the e~~Exemplary methods for preparing a DNA chip may include: a method of consecutively synthesizing DNA probes directly onto a substrate by using photolithography (US Patent No. 5,405,783 and so on); or a method for supplying synthesized DNA or synthesized cDNA

10 (complementary DNA) onto a substrate and ~~being bound~~ binding it thereto (US Patent No. 5,601,980, Japanese Patent Laid-Open No H11-187,900 (1999), an article ~~from in~~ "SCIENCE", Vol. 270, pp. 467 (1995) and so on).

[0004] In general, the biochip ~~are is~~ formed by using one
15 of the two methods described above, and when the thus-formed biochip is used for the applications described above, it is critical to know quantities, i.e., densities, of biological ~~related materials~~ used for forming probes that are included in ~~respective matrix,~~ matrices for the
20 purpose of ensuring the credibility of the analysis, i.e., the quantification or the reproducibility of the analysis. Further, it is also critical to know what type of matrix dimension (i.e., shape, size or condition) is provided to the matrix existing thereon (i.e., imaging),
25 purpose of assuring the quantification-ability or the reproducibility of the analysis. In addition, as described later, if there is no physical address for indicating the

expected position of a ~~respective matrix to be located on~~
the substrate that is employed for forming chips, an
additional problem may ~~be occurred~~. More specifically,
when the biochip is formed by using a method of supplying
5 fine droplets of a probe solution thereto via the ink jet
method, for example, an absence of the physical address
thereon may lead to an unclear determination ~~on~~ of the
position of the probe portion ~~where~~ when the analysis is
~~now~~ conducted on the biochip, depending on employed method.

10 In such case, the detection means itself must also ~~function~~
~~as enableing~~ a clear determination of the matrix position.

[0005] However, the probe on the biochip exists
principally as a monolayer or less, and in general, the
analysis of the biological ~~related materials~~ including the
15 clear determination of the matrix position requires ~~the~~
highly sensitive surface analysis techniques.

[0006] One of the known highly sensitive surface analysis
techniques that ~~satisfies~~ the aforementioned requirements
may be a method of using stable isotope labeled probes.

20 However, this method ~~contains~~ has various disadvantages
from the viewpoint ~~in view of applying~~ general purpose
usage, ~~that is,~~ Specifically the method requires a
complicated labeling method, ~~and the method requires as~~
well as special facilities and special equipments, ~~since~~
25 because the employed isotope itself may be a source of a
radioactive ~~pollution~~ emission.

[0007] Another method may be ~~a method~~ that of labeling the probe with a fluorescent label, or alternatively, ~~a method~~ that of labeling a specific material that specifically binds to the probe with a fluorescent label and then

5 binding it to the probe, which is known as a fluorescent-hybridization method for the DNA chip. However, ~~the~~ such a method also ~~contains~~ has various problems ~~against~~ with respect to achieving higher quantification-ability, such as a problem of the chemical stability of the fluorescent dye
10 used for labeling, a problem of the fluorescent quenching, a problem of the nonspecific adsorption of the fluorescent dye onto the substrate surface, or additionally the problem of the quantification-ability (i.e., stability, reproducibility) of the specific binding-ability (i.e.,
15 hybridization), ~~and~~ Thus, there are a number of problems for quantitatively detecting the amount of the existing probe itself.

[0008] Other highly sensitive surface analysis methods that are capable of being employed for analyzing general
20 detection objects include the ATR method that utilizes FT-IR ~~method~~ (Fourier Transform Infra Red Spectroscopy), XPS ~~method~~ (X-ray Photoelectron Spectroscopy) and so on.

However, these methods do not involve sufficient sensitivity for the quantitative analysis of the probe ~~of~~
25 ~~the~~ on a biochip, i.e., a biological ~~related~~ material, or imaging thereof. In particular, when a general purpose glass is employed as a substrate for producing the biochip,

these methods are not available ~~methods~~, ~~since because~~ the absorption due to the glass substrate itself adversely affects the analysis results when the FT-IR (ATR) method is employed, for example, or ~~since the charge-up because a~~
5 charge-up occurred on the glass, which is an electrically insulating material, adversely affectings the analysis ~~results when~~ the XPS method is employed.

[0009] Yet another highly sensitive surface analysis method that is capable of being employed for analyzing
10 biological related materials may be a DNA detection method utilizing the laser RIS (Resonance Ionization Spectroscopy) method, which is disclosed in United States Patent No.

5,821,060. In this method, the specimen surface is irradiated with laser or ion beams mentioned below, and the
15 generated portion is irradiated with a laser beam having a wavelength that is equivalent to ionization energy of a specific element, so that the specific element is ionized and emitted from the specimen surface and the emitted ionized element is detected. Disclosed methods for

20 releasing the element from the specimen surface may be a method utilizing a laser beam (laser ablation) or a method utilizing ions (ion sputtering). However, these methods have a technical limitation in ~~which that~~ only a limited number of elements ~~are possible to can~~ be detected.

25 [0010] Yet another highly sensitive surface analysis method may be dynamic SIMS (Secondary Ion Mass Spectrometry), in which an organic compound is decomposed

to smaller fragment ions or to particles during the process of generating a secondary ion. Thus, the amount of the information on the chemical structures obtained from the mass spectrum is not sufficient. ~~and thus~~ Thus, the method is not generally suitable for general purposes, since because the obtained information is not sufficient for the analysis of organic compounds such as, for example, nucleic acid-related materials having only ~~common~~ four common bases.

[0011] On the other hand, the time of flight secondary ion mass spectrometry (TOF-SIMS), which is also known as another technique of the secondary ion mass spectrometry, is an analysis method for investigating what types of atoms or molecules ~~are existing~~ on the uppermost surface of a solid specimen. ~~and the~~ This method has the following advantages: having ~~a detection~~ an ability for detecting a trace amount of a component of 10^9 atoms/cm² (equivalent to $1/10^5$ of ~~the~~ all atoms existing in one atomic layer of the uppermost surface); being applicable to both organic and inorganic compounds; being capable of detecting all types of elements and compounds that existing on the surface; and being ~~available of~~ able to image secondary ions from materials that are existing on the surface of the specimen.

[0012] Here, the principles of the time of flight secondary ion mass spectrometry will be described as follows.

[0013] ~~At~~In high vacuum ~~condition~~, a high speed pulsed ion beam (primary ion) irradiated to a surface of a solid specimen causes sputtering ~~phenomenon~~, in which a structural components of the surface ~~are~~is emitted into the vacuum. Ions (secondary ions) having positive or negative charges generated during this process are accelerated into a mass spectrometer, where they are mass-analyzed by measuring the travel time from the specimen surface to a detector. In the sputtering process, various ions having a variety of masses are generated depending on the chemical components of the surface of the specimen, and the ions having a smaller mass fly faster and, on the contrary, ions having a larger mass fly slower, within a constant electrical field. Thus, detecting the time ~~taken~~ elapsed from the generation of the secondary ions to the arrival of the generated ions to the detector (i.e., time of flight) provides an analysis of the mass of the generated secondary ions.

[0014] On the other hand, in the dynamic-SIMS method, organic compounds are decomposed to small fragment ions or particles during the ionization process as stated above and thus Thus, information on the chemical structure obtained from the mass spectrum, e.g., mass range, is limited. On the contrary, in the TOF-SIMS method, the structures of the organic compounds can be directly obtainable from the mass spectrum with a wide mass range, ~~since the extremely~~ because a much smaller amount of the

primary ions is necessary in the TOF-SIMS method, so that
while the organic compounds are ionized, they with
substantially ~~retaining~~ retain their chemical structure.
In addition, the information on the uppermost layer (within
5 a depth of several angstroms) of the object can be
selectively obtained ~~able~~ as only the secondary ions
generated in the uppermost solid surface are emitted into
the vacuum.

[0015] The TOF-SIMS apparatus that employs the principle
10 of the measurements described above is generally classified
~~to as~~ as a sector-type apparatus and a reflectron-type
apparatus. One of the differences between these two types
is ~~on~~ in the manner of electrically grounding of a holder
that fixes an object to be analyzed. In the sector-type
15 apparatus, the generated ions are led to the mass
spectrometer by applying positive or negative voltage of
several kV to the specimen-fixing holder, ~~and on the~~
~~contrary,~~ in the reflectron-type apparatus, the
specimen-fixing holder is grounded and the secondary ions
20 are led to the mass spectrometer by applying positive or
negative voltage of several kV to several-ten kV to an
extracting electrode for the secondary ions.

[0016] The TOF-SIMS method often utilizes positive primary
ions, and both positive secondary ions and negative
25 secondary ions are generated regardless of the polarity of
the utilized primary ions. Also, regardless of the
polarity of the utilized primary ions, the amount of the

secondary electrons that are generated by irradiating the primary ions is greater than the primary ions ~~in~~under the general measurement conditions, so that the surface potential tends to be positive, ~~and in~~In turn, when the positive charge accumulates beyond a certain level (i.e., charge-up condition), the excessive positive charge may disturb the quantitative measurements. In considering the apparatus configurations in relation with the charge-up condition, the measurements of the negative secondary ions from the insulator material by using the sector-type apparatus can cause the highest positive-charge accumulation, ~~(because all of the generated secondary electrons are directed toward the extracting electrode for the (negative) secondary ions, in which~~wherein the extracting electrode is applied with the above-mentioned positive voltage is applied to the extracting electrode.

[0017] In order to neutralize the positive charge caused by the above-mentioned charge-up condition, both the sector-type apparatus and the reflectron-type apparatus may often be equipped with a pulse-type electron gun for neutralizing the charge. A specific method for neutralizing the charge by using the pulse-type electron gun may include a step of applying the electron beam from the above-mentioned pulse-type electron gun onto the object to be analyzed for a constant duration irradiating primary ions (sub-nanosecond pulse to several nanosecond pulse) and before irradiating the primary ions for the next process of

generating secondary ions. Here, while the electron beam is ~~irradiating~~ irradiated by the pulse-type electron gun onto the object to be analyzed, the application of the voltage to the object holder (for the sector-type

5 apparatus) or to the secondary ion extracting electrode (for the reflectron-type apparatus) ~~is~~are-stopped, and the holder or the electrode ~~are~~is grounded, respectively.

[0018] The above-mentioned method of neutralizing the charge often relieves (or compensates for) the ~~charged-up~~ accumulated positive charge, enabling the analysis of the insulator material. Here, when the negative secondary ions are measured for the insulator material by using the sector-type apparatus, the insulator is most-considerably and positively charged, and thus the margin of the charge-
10 neutralization in this type of measurement is the narrowest. In ~~any way, in order~~ to prevent the ~~charging~~ charge-up, using the reflectron-type apparatus, in which the object holder is constantly electrically grounded ~~constantly~~, is (in general) more advantageous than using
15 the sector-type apparatus. In particular, when the object to be analyzed has a lower electric conductivity (in other words, higher electric resistivity or a lower dielectric constant), e.g., glass and the like, a reflectron-type apparatus is more suitable for carrying out the
20 quantitative measurements.

[0019] Regardless of ~~employing whether a~~ reflectron-type apparatus or a sector-type apparatus is employed, the TOF-

SIMS method is the analysis method of a considerably higher sensitivity, ~~so that t~~ This method enables the analysis of an object ~~to be analyzed of and is~~ less influential ~~influenced with~~ by a charging charge-up, e.g.,

5 oligonucleotide formed in a single molecular film level on a gold substrate having better electric conductivity.

(Proceeding of the 12th International Conference on Secondary Ion Mass Spectrometry, 951 (1999)). Further, an evaluation conducted by the present inventors shows that,

10 by conducting the process of preventing the charging-up, ~~the biological-related~~ materials such as oligonucleotide bound to the substrate surface ~~of~~ with a higher dielectric constant, such as a glass substrate, can be in-situ analyzed by irradiating the primary ions at a spot ~~having a~~
15 ~~diameter of several μ m level~~ in diameter when the analysis is conducted by an individual spot measurement.

[0020] However, the evaluation conducted by the present inventors also shows that when the two-dimensional secondary ion image was to be obtained by sequentially

20 scanning the primary ion beam having a beam diameter of 5 μ m in a constant direction, like the scanning line of ~~a~~ the TV receiver (i.e., raster scanning), onto the substrate of a higher resistivity across a ~~certainly wide~~ area, e.g., the area ~~of that is~~ 500 μ m x 500 μ m, a good

25 image was not obtained because of considerable influence of the ~~charging~~ charge-up.

SUMMARY OF THE INVENTION

[0021] The present invention provides a solution for the
aforementioned problems. The present invention provides a
measurement method, which enables one to obtain a two-
5 dimensional image with better quantitative-ability by
suppressing the influence of the ~~charging-up~~, charge
accumulation when the two-dimensional secondary ion image
is obtained for a biological-~~related~~ material fixed on a
substrate having high resistivity by utilizing a TOF-SIMS
10 method ~~in-over~~ a ~~certainly~~-wide area.

[0022] The present inventors have actively ~~involved~~
investigated ~~ions~~ ~~for~~ the above-mentioned problems, i.e.,
looked ~~ing~~ for a solution for suppressing the influence of
the ~~chargeing~~-up when two-dimensional imaging is conducted
15 via the TOF-SIMS method for a relatively large area of the
portion of a biochip that includes a biological-~~related~~
material formed on a substrate ~~of~~ having a relatively high
resistivity, ~~and~~ ~~the~~ The present inventors have found ~~from~~
~~results of our investigations provided that~~ a two-

20 dimensional image having a considerably high positioning
resolution-ability can be obtained by the procedure, in
which the pulsed primary ion beam is irradiated at a spot,
and the pulse-wise spot-applications of the primary ion
beam and the simultaneous detection of the secondary ion
25 generated from the irradiated primary ion beam ~~are~~
proceeded along with a discontinuous scanning pattern, and
eventually ~~the results of~~ these secondary ion measurements

results ~~is~~ are reconstructed into a two-dimensional image
in line with the aforementioned discontinuous scanning
pattern. Further, the present inventors have also
confirmed that, when the pulsed primary ion beam is
5 irradiated along with the aforementioned discrete pattern,
the chargeing-up of some insufficiently charge-neutralized
spots has dissipated until the detection of the secondary
ion for the adjacent spots is conducted, ~~and t~~ Therefore,
the present invention has been ~~made~~ achieved on the this
10 ~~basis of these knowledge.~~

[0023] That is, a ~~method for acquiring information from~~
~~the biochip~~ according to the present invention, ~~may be a~~
method for acquiring information in relation to a biochip,
which includesing a substrate and a plurality of
15 biological-related materials disposed on a surface of the
substrate, from the surface of the biochip using time of
flight secondary ion mass spectrometry, includesing at
least the steps of:

irradiating a pulsed primary ion beam on the surface of
20 the biochip in a discontinuous pattern, the surface of the
biochip having the biological-related material disposed
thereon, and the primary ion beam having a spot size of an
area that is a much smaller area than ~~an area~~ the one to be
measured on the surface of the biochip;

25 conducting mass-analysis of secondary ions via time of
flight, the secondary ion being generated by irradiating the
pulsed primary ion beam; and

reconstructing analyzed results obtained by conducting the mass-analysis to form a two-dimensional distribution information on the basis of the pattern of the applying primary ion beam in a pulse manner.

5 [0024] Further, a method for analyzing components ~~of a~~ on a biochip surface according to the present invention may be a method for analyzing components of a biological-related material disposed on a biochip, ~~in relation to the biochip~~ which includes a substrate and a plurality of biological-related materials disposed on a surface of the substrate,
10 ~~from the surface of the biochip~~ using time of flight secondary ion mass spectrometry, including at least the steps of:

irradiating a pulsed primary ion beam on the surface of
15 the biochip in a discontinuous pattern, the surface of the biochip having the biological-related material disposed thereon, and the primary ion beam having a spot size area that is ~~of much smaller area~~ than an area to be measured on the surface of the biochip;

20 conducting mass-analysis of secondary ions via time of flight, the secondary ion being generated by irradiating the pulsed primary ion beam;

reconstructing analyzed results obtained by conducting the mass-analysis to form a two-dimensional distribution
25 information on the basis of the pattern of the irradiating pulsed primary ion beam; and

conducting component-analysis of the biological-related material of a necessary portion contained in the obtained two-dimensional image on the basis of the mass spectrum information of the necessary portion.

5 [0025] In addition, the present invention also provides an apparatus adopted to be used for acquiring information from the above-mentioned biochip surface, that is, an apparatus for acquiring information from the biochip surface according to the present invention ~~is~~ may be an apparatus
10 for acquiring information in relation to a biochip including a substrate and a plurality of biological-related materials disposed on a surface of the substrate from the surface of the biochip using time of flight secondary ion mass spectrometry, including at least:

15 a device for irradiating a pulsed primary ion beam on the surface of the biochip in a discontinuous pattern, the surface of the biochip having the biological-related material disposed thereon, and the primary ion beam having a spot size of a much smaller area than an area to be measured
20 on the surface of the biochip;

a device for conducting mass-analysis of secondary ions via time of flight, the secondary ion being generated by irradiating the pulsed primary ion beam; and

a device for reconstructing analyzed results obtained
25 by conducting the mass-analysis to form ~~a~~-two-dimensional distribution information on the basis of the pattern of the irradiating pulsed primary ion beam.

[0026] Further objects, features and advantages of the present invention will become apparent from the following description of the preferred embodiments with reference to the attached drawings.

5

BRIEF DESCRIPTION OF THE DRAWINGS

[0027] Figs. 1-A, 1-B, 1-C and 1-D are images of the results of imaging according to Example 2, showing the imaging results obtained by reconstructing the data on the basis of PO_2^- ion (Fig. 1-A); PO_3^- ion (Fig. 1-B); PO_2^- ion + PO_3^- ion (Fig. 1-C); and (thymine-H) $^-$ ion (Fig. 1-D);

10

[0028] Fig. 2 is a graph showing the results of mass spectrum employed for obtaining the results of component analysis conducted in Example 2; and

15

[0029] Fig. 3 shows images prepared from the results of Example 8, and the images shown in the upper row are obtained by using Ga^+ , and the images in the lower row are obtained by using Au_3^+ .

DETAILED DESCRIPTION OF THE INVENTION

20

[0030] The present invention will be fully described in detail as follows.

25

[0031] The method according to the present invention is characterized in irradiating pulsed primary ions on the basis of the discontinuous scanning pattern for acquiring the images via TOF-SIMS, not based on the above-mentioned raster scanning, and also characterized in carrying out the

imaging by reconstructing the respective mass analysis results obtained by respective discrete pulse-application on the basis of the pattern of the discontinuous pulse-application of the primary ion. The technique of scanning
5 in the discontinuous scanning pattern enables imaging ~~the~~ of a relatively large area of the surface of the biochip that includes biological-related materials formed on the substrate having a relatively high resistivity.

[0032] The discontinuous scanning pattern may be any
10 pattern that enables avoiding the influence of the ~~chargeing-up, and~~ A typical discontinuous pattern may be a random pattern or a specifically programmed pattern. In such a case, although ~~an~~ overlapping of ~~an~~ unit (hereinafter called "pixel") being irradiated with primary
15 ion beam (having same shape as the shape of primary ion beam) with the adjacent pixel may be permitted, the overlapping of the pixels is not preferable, ~~since the overlapping because it~~ may cause a duplicated irradiation for an identical point in one scanning, so that the
20 obtained data does not reflect the actual value. Thus, if a random number is employed by the computer for generating ~~the a random scanning pattern of the scanning~~, the employed random number may preferably be one that is capable of providing a uniform probability of the generation scan
25 across the area being irradiated. Also, a programmed specific pattern described above may optionally be used, if necessary. The programmed specific pattern described above

may preferably have discrete scan path tracks, each of which is sufficiently discrete or separated to avoid the chargeing-up problem. If the scan path tracks of the programmed specific pattern are sufficiently discrete, an
5 effect equivalent to the one obtained by employing the random scanning can be expected by employing the programmed specific pattern. However, if the intervals between the discrete scan path tracks are short, or more specifically, for example, if the irradiation is carried out onto
10 alternate pixels, or, in other words, if the irradiated pixels are relatively closely disposed, the influence of the chargeing-up cannot sufficiently be avoided. Thus, when the above-mentioned "programmed specific pattern" is employed, the scan path tracks of the pattern may
15 preferably be designed to be sufficiently discrete.

[0033] When an image is formed by using a mass spectrum of the thus-obtained respective pixels, reconstructing ~~of the~~ data in the order of the measurements of the respective pixels may not provide ~~the~~ a suitable image that
20 appropriately reflects the actual condition, ~~since~~ because the scanning of the primary ion beam is carried out ~~with~~ in the discontinuous pattern, i.e., random pattern, specifically programmed pattern and so on. In such a case, the present invention provides ~~the~~ a suitable image that
25 appropriately reflects the actual conditions, by storing the irradiation pattern of the primary ion beam and

reconstructing the obtained data on the basis of the stored irradiation pattern.

[0034] The combination of the discontinuous application of the primary ion beam and the reconstructioning of the

5 obtained data according to the present invention is considerably advantageous in the measurement using ~~the~~ a substrate having a higher resistivity in which the measurement is considerably influenced by the chargeing-up,

~~and on~~ On the other hand, the combination according to

10 the present invention may not be fully advantageous in reality in the measurement using the substrate having a lower resistivity in which ~~the~~ suitable imaging can be carried out by using ~~the~~ ordinary raster scanning, ~~since~~ because the combination of the discontinuous scanning and

15 the reconstructioning of the data requires a longer period of time ~~to~~ for carrying out ~~the reconstructing of the data~~ than the ordinary raster scanning. In order to fully

provide the advantages thereof the invention, the scanning

technique may be selected depending on the resistivity of

20 the substrate to be used. For example, the range of the resistivity of the materials for the substrate, in which the discontinuous scanning is considerably advantageous, is a volumetric resistivity of not less than 10^{10} ohm·cm

(300K).

25 [0035] The volumetric resistivity of the substrate being preferably used for the substrate of the biochip may ~~be~~ not be less than 10^{10} ohm·cm (300K), and such a substrate is the

most suitable for applying the imaging method ~~of imaging~~ according to the present invention.

[0036] The species of the primary ion for the use in the present invention may preferably be a gallium ion (Ga^+) or a cesium ion (Ce^+), and, optionally, an Au ion (Au^+) and the like, in view of ionization efficiency, mass analysis resolution and so on. Here, the Au ion is more preferably used, because it ~~it~~ provides ~~providing~~ the mass analysis with a considerably higher sensitivity. In such a case, the available ion is not limited to the Au ion. ~~but an Au_2 ion and an Au_3 ion may be also used.~~ ~~and~~ ~~the~~ The sensitivity of the measurement often increases by selecting the Au ion. A greater increase is achieved ~~much increases~~ by selecting the Au_2 ion (Au_2^+). ~~A and much greater more increases is achieved by selecting the Au_3 ion (Au_3^+), thus presenting more preferable measurements.~~

[0037] When the imaging is carried out by using TOF-SIMS, the measurement conditions of mass analysis resolution, area for analysis and time for analysis are not uniquely determined, ~~since~~ because the conditions are closely and mutually related to pulse frequency of the primary ion beam, energy of the primary ion beam, pulse width of the primary ion beam, and the data handling ability of the computer employed for ~~using~~ the image processing. However, each ~~value~~ of these conditions should be within a range for enabling the analysis.

[0038] In view of the availability of the analysis, the pulse frequency of the primary ion beam used in the present invention may preferably be in the range from 1 kHz to 50 kHz, the energy of the primary ion beam may preferably be in the range from 12 keV to 25 keV, and the pulse width of the primary ion beam may preferably be from 0.5 ns to 10 ns.

[0039] In order to improve the measurement accuracy, the measurement should be completed in a short period of time (an order of several --tens of seconds to several --tens of minutes) while maintaining the high mass resolution. For ~~and for~~ this reason, the measurement may preferably be carried out without using a highly-focused primary ion beam, ~~for the purpose of to completeing~~ the measurement in a short period of time. More specifically, it is not necessary to highly focus the aperture diameter of the primary ion beam ~~is not necessary to be highly focused to a~~ sub-micron level by a relatively complicated operation. ~~but~~ It may preferably be focused ~~to~~ at the level ranging from 1 μm to 10 μm by a relatively simple operation. This ~~range of the diameter range~~ is preferable, ~~in~~ considering that the size of the respective matrix (also called "dot" or "spot") on the biochip to be analyzed according to the present invention normally has a circular shape having a diameter ~~of~~ from 10 μm to 100 μm , or a rectangular shape ~~having a dimension of that ranges~~ from 10 μm x 10 μm to 100 μm x 100 μm .

[0040] The area for scanning is not uniquely determined, ~~since the area of scanning~~ because it is related to other factors as mentioned above, However, -but- preferably, this area has a circular shape having a diameter within a range
5 from 50 μm to 500 μm , or ~~the a~~ a rectangular shape ~~having a~~ dimension within a that ranges from 50 μm x 50 μm to 500 μm x 500 μm .

[0041] The number of the irradiating primary ion beams, i.e., the pixels, in one specific scanning process depends
10 on the size of scanning area, the diameter of the primary ion beam, the level of the overlapping of the pixels, or the frequency of the primary ion beam or the scanning time for one scanning, ~~and the t~~ These conditions automatically determine the number of the pixels composing the secondary
15 ion image. In this sense, the secondary ion image may be composed of pixels within a range from 56 x 56 pixels to 1024 x 1024 pixels.

[0042] The outer size of a generally used biochip may be, for example, 1 cm x 1 cm, 1 inch x 1 inch (25.4 cm x 25.4
20 cm) or slide glass size (e.g., 26 mm x 76 mm), and the matrix may be disposed within this size. The sizes of the scanning areas illustrated above are not sufficiently wide for scanning across such ~~these sizeds of the biochip for the~~ to imageing ~~of the entire surface thereof~~. In such a case,
25 a process of positional scanning (in general, called "stage scanning", as a stage having a substrate thereon is scanned in this scanning process) of the substrate may be

optionally employed in addition to the primary ion beam
~~scanning~~ to scan a wider area of the surface, as required.

In this case, a longer period of time for analysis is
required if a wider area is scanned. However, since the

5 matrix does not usually cover ~~across~~ the entire surface of
the biochip, the necessary area for the analysis may be
selected depending on the requirement, and the scanning

area may preferably be ~~a circular shape having~~with a
diameter of 1 mm or greater or a rectangular shape of a

10 dimension of 1 mm x 1 mm or ~~broader~~larger, or more

preferably, ~~a circular shape having~~with a diameter within a
range from 10 mm to 30 mm.

[0043] As described above, the main feature of the present
invention is the imaging of the biochip via TOF-SIMS. ~~In~~

15 ~~the reverse view thereof~~, From a different perspective, the

imaging of the present invention is carried out on the
basis of the mass data of the fragments, which can be

detected, measured and analyzed by using TOF-SIMS. ~~In~~

~~other view thereof~~ From yet another perspective, the mass

20 spectrum data can be principally extracted from the portion
(or the pixel) in which the mass data of the biochip for

imaging is detected. The present invention includes the
component analysis of the portions in which the imaging is

carried out and the positions thereof are specified. The

25 imaging of the specified portions of the actually prepared
biochip via this method enables the determination of the

positions and the shapes, and the component analysis of the positions.

[0044] The biological-related material disposed on the biochip, which is imaged or component-analyzed according to the present invention, is not particularly limited and may be any material as long as the material can be imaged or component-analyzed according to the TOF-SIMS method of the present invention. ~~and a~~ According to the evaluation of the present inventors, nucleic acids and proteins are preferable for ~~being the~~ analysised. ~~The e~~Examples of the nucleic acids may include DNA such as

oligodeoxynucleotides, polydeoxynucleotides, cDNA (complementary DNA) and so on, RNA, such as mRNA (messenger RNA), tRNA (transfer RNA), rRNA (ribosomal RNA) and so on, and nucleic acid analogues being typically represented by peptide nucleic acid (PNA), the molecular bone of which comprises peptides. Examples of the proteins may include oligopeptides, polypeptides, enzymes, antibodies and so on.

[0045] The ~~existing form of the~~ biological-related material on the substrate may be in any form. However, it is ~~but may preferably be a form of being~~ covalently bonded ~~with to~~ the substrate surface, in view of the form of the use of the biochip (for example, the form of the hybridization in the case of the DNA chip) and the stability of, for example, the level of ionization during the analysis using TOF-SIMS method. Various methods are known for forming the covalent bond ~~of between~~ the

biological-related material ~~with~~ and the substrate surface,
and ~~the~~ a suitable method can be selected from these known
methods. An example of the method of forming the covalent
bond is disclosed in the Japanese Patent Laid-Open No. H11-
5 187,900 (1999).

[0046] Also, methods for sequentially synthesizing the
nucleic acids and proteins on the solid phase materials are
known for one form of forming the covalent bond, and these
methods can be employed for preparing the biochip that is
10 the object of the method according to the present
invention.

[0047] Further, the method of ~~covalent~~ covalently bonding
the biological-related material ~~with~~ to the substrate may
also include ~~the~~ a method of covalently -bonding a first
15 functional group included in the biological -related
material, e.g., a nucleic acid or a protein, with a second
functional group bonded to the surface of the substrate, by
supplying the biological-related material onto the
substrate, ~~in which~~ wherein the second functional group is
20 capable of reacting with the first functional group to form
the covalent bond therebetween. The method of supplying
the biological-related material onto the substrate for
employing in the present invention may include the ink-jet
method typically including the known piezo-jet method and
25 the thermal jet method. ~~The~~ Japanese Patent Laid-Open No.
H11-187,900 (1999) also discloses ~~the~~ a method of supplying
a DNA probe onto ~~the~~ a substrate by the thermal jet method.

[0048] It is necessary to detect the fragment ions that ~~is~~
are specific to the above-mentioned biological-related
materials as secondary ions, ~~for~~ in order to carrying out
the imaging and the component analysis of the biochip via
5 the TOF-SIMS method, ~~and the~~ The fragment ion may be any
ions, as long as ~~the it ion~~ is specific to the biological-
related material and is capable of being detected by the
TOF-SIMS method.

[0049] The non-limiting examples of the biological-related
10 materials and ~~the~~ specific fragment ions ~~will be~~ are
~~described in the followings~~ below.

[0050] When the biological-related material is ~~the a~~
nucleic acid, ~~the material it~~ must have the backbone
consisting of diester phosphates, ~~and.~~ Therefore, the
15 fragment ions of the nucleic acid may include P-, PO-, PO2-
and PO3-, which are the fragment ions of the above-
mentioned backbone of diester phosphate, and these ions are
capable of being detected via the TOF-SIMS method.

[0051] Further, when the nucleic acid is DNA, the material
20 should include ~~four bases of~~ adenine, thymine, guanine and
cytosine, and thymine is replaced with uracil in the case
of RNA. Also, PNA, an exemplary nucleic acid analogue,
should include ~~four bases of~~ adenine, thymine, guanine and
cytosine. Thus, fragment ions of these bases, i.e.,
25 (adenine-H)-, (thymine-H)-, (guanine-H)-, (cytosine-H)- and
(uracil-H)- can be employed for the secondary ions.

[0052] PNA also has a backbone that constitutes peptides, ~~and~~ Thus, fragment ions of peptides, such as CNO- ion or CN- ion, can be employed for the detection via the TOF-SIMS method.

5 [0053] When the biological-~~related~~ material to be detected is a protein, the fragment ions of the peptides can be employed, because ~~since~~ the backbone of the protein ~~contains~~ stitutes peptides, as in the case of PNA. In addition, fragment ions derived by the residual group of
10 each amino acid can also be employed. Here, the efficiency of the detection for proteins is generally lower than the efficiency for nucleic acids, ~~since~~ because the mass spectrum intensity of one species derived by one amino acid of protein, which consists of more than 20 types of amino
15 acids, is lower than the mass spectrum intensity of one species derived by one base of nucleic acids, such as DNA, RNA and PNA, which consists of four bases.

[0054] In the method for acquiring information, a TOF-SIMS apparatus for the use in performing two-dimensional imaging
20 and component analysis may be any type of TOF-SIMS apparatus, as long as the apparatus is capable of performing detection, two-dimensional imaging and composition analysis. Here, the reflectron type apparatus, in which the holder for fixing the substrate is usually
25 grounded, is preferably employed, ~~in view of the purposes for~~ due to the need to effectively ~~reducing~~ the influence of the chargeing-up that occursred on the substrate during

the handling of the insulator material, as stated
~~before~~above.

EXAMPLES

[0055] The present invention will be described more
5 specifically, by illustrating examples.

~~(EXAMPLE~~ Example 1) Preparation of a nucleic acid probe
chip by using a dT40 probe

[0056] A nucleic acid probe was prepared by using quartz
glass, similarly as in the method described in the Japanese
10 Patent Laid-Open No. H11-187,900 (1999).

(1) Washing of the substrate

[0057] A 25.4 mm x 25.4 mm synthesized quartz substrate
~~having a dimension of 25.4 mm x 25.4 mm~~ was ~~disposed in~~
placed on a rack, and ~~the substrate was~~ immersed in a
15 ~~detergent solution that contains~~ a detergent for
ultrasonic washing (GPIII, commercially available from
BRANSON) diluted to 10% with pure water for one night.
Then, the substrate was ultrasonic-washed in the detergent
solution for 20 minutes, and ~~after that then~~ substrate was
20 washed with water to remove the detergent. After being
rinsed with pure water, the substrate was further
ultrasonic-washed within a container containing pure water
for 20 minutes. Next, the substrate was immersed in an
aqueous solution of 1N sodium hydroxide that was pre-heated
25 to 80°C for 10 minutes. Sequentially, the substrate was

washed with water and further washed with pure water, and the washed substrate was transferred for further ~~to the next~~ processing without conducting a drying process.

(2) Surface treatment

5 [0058] An aqueous solution of 1%wt. of N- β -(aminoethyl)- γ -aminopropyltrimethoxysilane, KBM603 (commercially available from SHIN-ETSU CHEMICAL IND. CO. LTD.), which is a silane coupling agent having amino acids bonded thereto, was stirred at room temperature for 2 hours to achieve a
10 hydrolysis of the methoxy group contained in the molecule~~ar~~ of the silane compound. The ~~washed~~ substrate that was washed ~~in the process as~~ described in the above section (1) was then immersed into the aqueous solution of the silane coupling agent for 1 hour, and after that the substrate was
15 washed with pure water, and ~~the both~~ sides of the substrate ~~was were~~ dried by ~~being blowing~~ with nitrogen gas ~~to the both sides thereon~~. Next, the substrate was baked in an oven that was heated to 120°C, for 1 hour, and thereby,
20 the substrate.

[0059] Next, 2.7 mg of N-(Maleimidocaproyloxy)succinimide (commercially available from DOJINDO LABORATORIES, hereinafter called "EMCS") was dissolved into a solution of 1:1 (by volumetric ratio) of dimethyl sulfoxide (DMSO)/
25 ethanol to prepare a solution having a concentration of 0.3 mg/ml. The substrate, which had been treated via the

silane-coupling treatment, was immersed in the EMCS solution at room temperature for 2 hours to react the amino group, which is introduced to the substrate surface via the silane coupling treatment, with the succinimide group of EMCS. The reaction introduced a maleimide group derived from EMCS present existing on the substrate surface. The substrate was then picked up from the EMCS solution, was washed with the aforementioned DMSO/ethanol solution, was washed with ethanol, and then was dried by being blowing with nitrogen gas thereon.

(3) Synthesis of probe DNA

[0060] Single strand nucleic acid of base sequence No. 1 (40mer of dT) was synthesized, by ordering a DNA synthesis company (BEX CO. LTD.). Sulfanilic group (SH) was introduced to the 5' end of the single strand DNA of the base sequence No. 1, by using a thiol modifier (available from GLENN RESEARCH CENTER). After the DNA synthesis of DNA, the deprotecting and the recovering of DNA were carried out according to the ordinary methods, and DNA was purified by using HPLC. The series of the processing from the synthesis to the purification was conducted by the aforementioned DNA synthesis company.

[0061] Sequence No. 1

5' HS- (CH₂)₆-O-PO₂-O-TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT

TTTTTTTTTT 3'

(4) DNA discharge by using a thermal jet printer and binding of DNA to the substrate

[0062] The single--stranded DNA described in the above section (3) was dissolved into an solution, which contained
5 7.5%wt. of glycerin, 7.5%wt. of urea, 7.5%wt. of thioglycol, and 1%wt. of acetylene alcohol (under the product name of "ACETYLENOL EH", commercially available from KAWAKEN FINE CHEMICAL CO., LTD.), to obtain an eventual concentration of 8 μ m.

10 [0063] Meanwhile, a printer head ("BC-50", commercially available from CANON CO. LTD.) for a bubble jet printer ("BJF-850", commercially available from CANON CO. LTD.), which employs a bubble jet method that is one of the thermal jet methods, was altered so that the altered
15 printer head was capable of discharging several--hundred ml of the solution. The altered printer head was mounted to a discharge drawing device, which was also altered so as to be capable of discharging the solution onto the flat quartz substrate. Several--hundred ml of the above-mentioned DNA
20 solution was transferred into an altered tank of the printer head, and the EMCS-treated substrate was mounted to the discharge drawing device to carry out a spotting operation onto the EMCS-treated surface of the substrate. Here, the discharge rate during the spotting operation was
25 4 pl/droplet, the area of the spotting operation was 10 mm x 10 mm, and the spotting was carried out at 200 dpi for that area, i.e., the discharge was performed at a pitch of

127 μm . ~~In this~~ Under these conditions, the diameter of the spotted dot was approximately 50 μm .

[0064] After completing the spotting operation, the substrate was left in a humidifier chamber for 30 minutes so that the maleimide group of the substrate surface ~~was~~ would reacted with the sulfanilic group (SH) of the 5' end of the nucleic acid probe to fix the DNA probe thereon.

Then, the substrate was washed with ~~pure water~~, and stored in ~~the~~ pure water. The obtained DNA-combined substrate (DNA chip) was dried by being blown on with nitrogen gas, and was stored in a vacuum ~~deiceator~~ desiccator to be further dried, just before conducting the analysis via TOF-SIMS.

~~{Example 2}~~ Imaging and composition analysis via TOF-SIMS

(1) Operations

[0065] ~~Operations of t~~The imaging and the composition analysis for the DNA chip prepared in the above-mentioned Example 1 were carried out by using a "TOF-SIMS IV" apparatus, which is commercially available from ION TOF CO. LTD.

[0066] The apparatus and conditions used in this operation are listed below.

[0067] <primary ion>

primary ion beam: 25 kV, Ga^+ , 0.6 pA (pulse current), random scan mode;

pulse frequency of the primary ion beam: 2.5 kHz (400 μ sec./shot);

pulse width of the primary ion beam: 1 ns; and

beam diameter of the primary ion beam: 5 μ m.

5 <secondary ion: imaging was carried out by reconstructing the obtained data according to the application pattern of the primary ion beam>

detection mode for secondary ion: negative;

area for the measurement: 300 μ m x 300 μ m;

10 number of pixel in the secondary ion image: 128 x 128 pixels; and

number of integrating operation: 256.

(2) Measurement results

[0068] Fig. 1 shows the results of the imaging for the
15 typical ion species from the data obtained by analyzing the DNA chip prepared in the Example 1 using the "TOF-SIMS IV" apparatus under the conditions described above. Fig. 1-A and Fig. 1-B represent the results of imaging of the PO_2^- ion and the PO_3^- ion, respectively, both of which are the
20 fragment ions of DNA phosphate backbones. As can be seen from these two-dimensional images, it was confirmed that DNA existed was present on the DNA chip in a shape of spotted form deposited by using a bubble jet device (i.e., a substantially circular shape having a diameter of about
25 50 μ m, and the pitch between the dots being about 125 μ m). It is also possible to obtain a two-dimensional image by

using the sum of the PO_2^- ion and the PO_3^- ion, as shown in Fig. 1-C, as well as the imaging of one fragment ion species.

[0069] It is also possible to conduct ~~an~~ imaging by using
5 a $\text{C}_5\text{H}_5\text{N}_2\text{O}_2^-$ ion, ~~that~~ which is the fragment ion derived from the nucleic acid base, for example, as shown in Fig. 1-D, as well as one using the fragment ion of a phosphate backbone. Since the probe DNA used in the present example was a homo-oligomer of thymidylic acid, the detected
10 fragment ion derived from the nucleic acid base was only the $\text{C}_5\text{H}_5\text{N}_2\text{O}_2^-$ ion, i.e., (thymine-H) $^-$ ion.

[0070] Fig. 2 shows mass spectrum profiles for the inner portion and the outer portion of a dot included in the obtained images concerning the typical secondary ions. For
15 example, if the fragment ion is a SiH_3 ion, the ~~existence~~ presence of the ion is detected equally in either ~~of~~ the inner portion ~~and~~ or the outer portion of the dot, ~~since~~ because the SiH_3 ion is not specific to the DNA existing inside the dot. ~~On~~ To the contrary, if the fragment ion is
20 O_2^- , P^- , PO^- , PO_2^- , PO_3^- , CNO^- (derived from the nucleic acid base) and $\text{C}_5\text{H}_5\text{N}_2\text{O}_2^-$, which are specific to the DNA existing inside the dot, or, in other words, ~~which~~ if the probability of the ion existing inside the dot is higher than the probability of existing outside the dot, the
25 intensity of the detected ion strength for these ions ~~are~~ is stronger inside the dot than outside the dot. As seen in the results shown in Fig. 2, the use of the present

invention enables the component analysis of the portion, the position of which is determined, by conducting the two-dimensional imaging via the mass spectroscopy.

5 ~~{Example 3}~~ preparation of a nucleic acid probe array by employing 50mer probe containing mixed four types of nucleic acid bases, imaging and component analysis thereof

(1) Preparation of DNA chip

10 [0071] DNA chip was prepared with DNA of the following base sequence No. 2, in the procedure identical to the procedure described in ~~the~~ Example 1.

[0072] Sequence No. 2

5'HS-(CH₂)₆-O-PO₂-O-TGCAGGCATG CAAGCTTGGC ACTGGCCGTC
GTTTTACAAC GTCGTGACTG 3'

(2) Imaging and composition analysis via TOF-SIMS

15 [0073] Imaging and composition analysis for the DNA chip comprising DNA of the above-identified sequence No. 2 were conducted via the method and conditions identical to ~~that~~ these described in ~~the~~ Example 2.

20 [0074] The results ~~of the present Example~~ show that the imaging and the component analysis by the respective fragment ions of (adenine-H)⁻, (guanine-H)⁻ and (cytosine-H)⁻ can be conducted, as well as the imaging and the component analysis for the fragment ions for the phosphate backbone and the fragment ions, such as (thymine-H)⁻
25 described in ~~the~~ Example 2.

{Example 4} Preparation of RNA chip, imaging and component analysis thereof

(1) Preparation of RNA chip

[0075] RNA chip was prepared with RNA (U20) of the

5 following base sequence No. 3, ~~in the using a procedure~~
~~that is identical to the procedure one described in the~~
Example 1, except that all the preparation processes were
carried out ~~under the condition of being free of RNase that~~
is an RNA decomposition enzyme.

10 [0076] Sequence No. 3

5'HS-(CH₂)₆-O-PO₂-O-UUUUUUUUUU UUUUUUUUUU 3'

(2) Imaging and composition analysis via TOF-SIMS

[0077] Imaging and composition analysis for the RNA chip
comprising RNA of the above-identified sequence No. 3 were
15 conducted via the method and conditions identical to ~~that~~
~~those~~ described in ~~the~~ Example 2. Here, the RNA chip
substrate was maintained ~~to be in the condition of~~ RNase
free just until the TOF-SIMS analysis was started.

[0078] The results of the present Example show that the
20 imaging and the component analysis by the fragment ion of
(uracil-H)- can be conducted, as well as the imaging and
the component analysis for the phosphate backbone-derived
the fragment ions in ~~the~~ Example 2.

{Example 5} Preparation of PNA chip, imaging and component analysis thereof

(1) Preparation of PNA chip

[0079] PNA having the base sequence identical to the base
5 sequence of the DNA probe prepared in the Example 3
(referred to as Sequence No. 2') was synthesized, by
~~ordering a~~ DNA synthesis company (BEX CO. LTD.). Here,
cysteine, one of the amino acids, was bonded to the N end
(corresponding to the 5' end of nucleic acid) via a linker
10 described below. Since cysteine contains a (SH-) group in
the branch, PNA ~~is possible to~~ can bind with the maleimide
group ~~existing present~~ on the quartz substrate after its
surface is treated.

[0080] The PNA chip was prepared with PNA of ~~the~~ sequence
15 No. 2', ~~in the~~ using a procedure identical to ~~the~~ procedure
~~described in the~~ Example 1.

[0081] Sequence No. 2'

NCys-NH-(CH₂)₂-O-(CH₂)₂-O-CH₂CONH-TGCAGGCATG CAAGCTTGGC
ACTGGCCGTC GTTTACAAC GTCGTGACTG

20 (2) Imaging and composition analysis via TOF-SIMS

[0082] Imaging and composition analysis for the PNA chip
comprising PNA of the above-identified sequence No. 2' were
conducted via the method and conditions identical to ~~that~~
those described in ~~the~~ Example 2.

[0083] The results ~~of the present Example~~ show that the imaging and the component analysis by the respective fragment ions of (adenine-H)⁻, (thymine-H)⁻, (guanine-H)⁻ and (cytosine-H)⁻, derived from four bases that constitutes PNA, can be conducted. Here, since PNA has no phosphate backbone, no fragment ion derived from the phosphate backbone was detected. ~~On~~ To the contrary, the fragment ions derived from the peptide bonds ~~contained~~ in the backbone of PNA, for example, CNO⁻ ions and CN⁻ ions, were
5 detected.
10

~~{Example 6}~~ Preparation of protein chip, imaging and component analysis thereof

(1) Preparation of protein chip

[0084] A ~~P~~protein chip was prepared by fixing a protein ~~to~~
15 on a quartz substrate surface ~~in a different~~ using a method
different from the methods for preparing synthesized nucleic acid probes described in Examples 1-5. ~~7~~ and ~~more~~
specifically, bovine serum albumin (BSA: commercially available from SIGMA ALDRICH JAPAN) was used. Here, BSA
20 contains a cysteine residual group. ~~7~~ and thus ~~Thus, the~~
protein was bound to the substrate surface via the reaction of SH- of cysteine and the maleimide group on the substrate surface.

[0085] Spotting ~~operation~~ of a protein solution was
25 carried out as in ~~the~~ Example 1 to prepare the protein chip. Here, the conditions, such as the solvent ~~condition~~

and the BSA concentration during the discharging process of the BSA via the bubble jet, were ~~optimistically~~ accordingly adjusted.

(2) Imaging and composition analysis via TOF-SIMS

5 [0086] Imaging and composition analysis for the protein chip comprising the above-identified BSA fixed thereto were conducted via the method and conditions identical to ~~that~~ those described in ~~the~~ Example 2, except that the detection mode for the secondary ion was selected to be positive.

10 [0087] The results ~~of the present Example~~ show that the imaging and the component analysis by ~~the~~ several fragment ions of residual groups of amino acids can be conducted. Typical secondary ion species were: $C_4H_8N^+$ and $C_4H_6N^+$, which ~~that~~ are considered to be fragment ions derived by proline (Pro), CH_3N^+ , $C_2H_7N_3^+$, $C_4H_{10}N_3^+$, $C_4H_{11}N_3^+$ and $C_5H_8N_3^+$, that ~~which~~ are considered to be fragment ions derived by an arginine (Arg) residual group; and $C_9H_8N^+$, $C_{10}H_{11}N^+$ and $C_{11}H_8NO^+$, which ~~that~~ are considered to be fragment ions derived by a tryptophan (Trp) residual group. Further, $C_2H_6NS^+$ and CHS^+ , that ~~which~~ are considered to be fragment ions derived by a cysteine (Cys) residual group, were also detected. As can be seen from the results described above, the detection of the above-mentioned fragment ions, which are considered to be derived by amino acid residual groups, enables the

20

25 imaging of the protein disposed on the insulator substrate surface. When the protein having characteristic amino

residual groups is detected, an image equivalent to a two-
dimensional distribution of the protein can be created by
detecting the above-mentioned fragment ions. Further, a
combination of the image analysis and numerical analysis
5 for an image created by the respective above-mentioned
fragment ions, which are considered to be derived by
respective amino acid residual groups (e.g., digitalization
of the amount of the amino acids contained in the protein
~~are-is~~ conducted for a plurality of proteins is carried out
10 and then the resultant digitalized data are correlated with
the intensity of the above-mentioned fragment ions (i.e.,
image intensity)), can be carried out to obtain images (a
~~two--~~dimensional distribution image) of respective
proteins.

15 ~~{Example 7}~~

[0088] Imaging and composition analysis for the DNA chip
prepared in ~~the~~ Example 1 were conducted via the method and
conditions identical to that described in ~~the~~ Example 2,
except that the employed primary ion was Au⁺. The results
20 ~~of the present Example~~ show that the mass spectrum for the
respective ions detected in Example 2 can be obtained with
double--digit--higher sensitivity and ~~the~~ better imaging on
the basis of the mass spectrum with higher sensitivity--~~can~~
~~be obtained.~~

25 ~~{Example 8}~~ ~~p~~Preparation of a nucleic acid probe array by
employing 13mer probe containing mixed four types of

nucleic acid bases, imaging and component analysis thereof by using TOF-SIMS method with the primary ion species of Ga^+ and Au_3^+ .

(1) Preparation of DNA chip

5 [0089] A DNA chip was prepared with DNA of the following sequence No. 4, ~~in the~~ using a procedure identical to the ~~procedure that~~ described in the Example 1.

[0090] Sequence No. 4

5'HS-(CH₂)₆-O-PO₂-O- ACTGGCCGTC GTTTTACA 3'

10 (2) Imaging and composition analysis via TOF-SIMS

[0091] Imaging and composition analysis for the DNA chip comprising DNA having the above-identified sequence No. 4 were conducted by using Ga^+ and Au_3^+ for primary ions (apparatus employed for the present Examples was "TOF-SIMS
15 IV" commercially available from ION TOF CO. LTD). The conditions for measurements are listed below.

[0092] Case of using Ga^+ for primary ion species:

<primary ion>

primary ion beam: 25 kV, Ga^+ , 0.6 pA (pulse current), random
20 scan mode;

pulse frequency of the primary ion beam: 2.5 kHz (400 $\mu\text{sec./shot}$);

pulse width of the primary ion beam: approximately 1 ns; and beam diameter of the primary ion beam: 5 μm .

<secondary ion: imaging was carried out by reconstructing the obtained data according to the application pattern of the primary ion beam

detection mode for secondary ion: negative;

5 area for the measurement: 300 μm x 300 μm ;

number of pixel in the secondary ion image: 128 x 128 pixels; and

number of integrating operation: 256.

[0093] Case of using Au_3^+ for primary ion species:

10 <primary ion>

primary ion beam: 25 kV, Au_3^+ , 0.07 pA (pulse current), random scan mode;

pulse frequency of the primary ion beam: 5 kHz (200 $\mu\text{sec./shot}$);

15 pulse width of the primary ion beam: approximately 1 ns; and beam diameter of the primary ion beam: 5 μm .

<secondary ion: imaging was carried out by reconstructing the obtained data according to the application pattern of the primary ion beam>

20 detection mode for secondary ion: negative;

area for the measurement: 300 μm x 300 μm ;

number of pixel in the secondary ion image: 128 x 128 pixels; and

number of integrating operation: 281.

25 [0094] Fig. 3 shows the analysis results via TOF-SIMS

obtained by using Ga^+ and Au_3^+ according to the conditions described above. Fig. 3 includes the images for PO_2^- , PO_3^- ,

$C_4H_4N_3^-$, $C_5H_5N_2O_2^-$, $C_5H_4N_5^-$ and $C_5H_4N_5O^-$, which are the typical secondary ions obtainable in the TOF-SIMS analysis for the DNA probe array containing ~~mixed~~-four mixed types of nucleic acid bases, by using Ga^+ (shown in upper row) or by
5 using Au_3^+ (shown in a lower row). Here, the description "mc" refers the maximum value in a pixel, and "tc" refers to the total count number in the whole 128 x 128 pixels. As seen in these images, employing Au_3^+ provides a nearly double-digit higher sensitivity for PO_3^- ~~by nearly double~~
10 ~~digit,~~ and also provides a greater than a double-digit ~~much~~ ~~higher~~ sensitivity for the fragment ions derived from the four bases ~~by greater than double digit~~, as compared with to the case of employing Ga^+ (about 87-fold as reduced to the case of equivalent dosage, or about 20-fold as reduced
15 to the case of equivalent measurement time (as 0.12-fold decrease in the pulse current, and 2-fold increase in the pulse cycle)). Thus, it was found that the use of the Au_3^+ gun for the TOF-SIMS analysis of the biochip was considerably advantageous.

20 [0095] While the present invention has been described with reference to what are presently considered to be the preferred embodiments, it is to be understood that the invention is not limited to the disclosed embodiments. ~~On~~ To the contrary, the invention is intended to cover various
25 modifications and equivalent arrangements included within the spirit and scope of the appended claims. The scope of the following claims is to be accorded the broadest

interpretation so as to encompass all such modifications and equivalent structures and functions.

WHAT IS CLAIMED IS:

1. A method for acquiring information in relation to a device including a substrate and a plurality of materials disposed on a surface of said substrate from said surface of said device using time of flight secondary ion mass spectrometry, including at least the steps of:

irradiating pulsed primary ion beam on different positions of said surface of said biochip in a discontinuous pattern, and said primary ion beam having a spot size of smaller area than an area to be measured on said surface of said device;

conducting mass analysis of secondary ions via time of flight, said secondary ion being generated by irradiating said pulsed primary ion beam; and

reconstructing analyzed results obtained by conducting said mass analysis to form a two dimensional information on the basis of said pattern of said irradiating pulsed primary ion beam.

2. The method according to claim 1, wherein said discontinuous pattern is selected to be a two dimensionally random pattern.

3. The method according to claim 1, wherein said discontinuous pattern is selected to be a specifically programmed pattern.

~~4. The method according to claim 1, wherein an ion species of said primary ion beam is gold ion (Au^+ , Au_2^+ , Au_3^+).~~

5

~~5. The method according to claim 1, wherein the acquisition of information from the device surface is conducted by a combination of scanning of the primary ion beam and positional scanning of said substrate itself.~~

10

~~6. The method according to claim 1, wherein the device is a chip, on which biological-related materials are disposed.~~

15

~~7. The method according to claim 6, wherein said biological-related material is nucleic acid.~~

~~8. The method according to claim 7, wherein the nucleic acid is selected from the group consisting of DNA and RNA.~~

20

~~9. The method according to claim 8, wherein the DNA is selected from the group consisting of oligodeoxynucleotides, polydeoxynucleotides and cDNA (complementary DNA).~~

25

~~10. The method according to claim 6, wherein said biological-related material is PNA (peptide nucleic acid).~~

~~11. The method according to claim 6, wherein said biological-related material is protein.~~

5 ~~12. The method according to claim 7, wherein the secondary ion species generated by said primary ion beam includes at least species derived by the fragmentation and ionization of phosphate backbone derived from nucleic acid.~~

10 ~~13. The method according to claim 12, wherein the secondary ion species generated by said primary ion beam includes at least any one of P^- , PO^- , PO_2^- and PO_3^- .~~

15 ~~14. The method according to claim 8, wherein the secondary ion species generated by said primary ion beam includes at least species derived by the fragmentation and ionization of nucleic acid base.~~

20 ~~15. The method according to claim 14, wherein the secondary ion species generated by said primary ion beam includes at least any one of (adenine-H) $^-$, (thymine-H) $^-$, (guanine-H) $^-$, (cytosine-H) $^-$ and (uracil-H).~~

25 ~~16. The method according to claim 10, wherein the secondary ion species generated by said primary ion beam includes at least species derived by the fragmentation and ionization of peptide backbone.~~

~~17. The method according to claim 11, wherein the secondary ion species generated by said primary ion beam includes at least species derived by the fragmentation of amino acid residual group and species derived by the ionization of amino acid residual group.~~

~~18. The method according to claim 1, wherein said apparatus of time of flight secondary ion mass spectrometry for the use in the method is selected to be a reflectron type apparatus in which the measurement is carried out while said substrate is held in a condition of electrically grounded.~~

~~19. A method for analyzing components of a biological-related material disposed on a biochip in relation to the biochip, which includes a substrate, and a plurality of biological-related materials disposed on a surface of said substrate from said surface of said biochip using time of flight secondary ion mass spectrometry, including at least the steps of:~~

~~irradiating pulsed primary ion beam on said surface of said biochip in a discontinuous pattern, and said primary ion beam having a spot size of smaller area than an area to be measured on said surface of said biochip;~~

~~conducting mass analysis of secondary ions via time of flight, said secondary ion being generated by irradiating~~

~~said pulsed primary ion beam;~~

~~—— reconstructing analyzed results obtained by conducting
said mass analysis to form a two dimensional information on
the basis of said pattern of said irradiating pulsed primary
ion beam; and~~

~~—— conducting component analysis of the biological related
material of a necessary portion contained in the obtained
two dimensional image on the basis of the mass spectrum
information of said necessary portion.~~

~~20. An apparatus for acquiring information in relation
to a biochip including a substrate and a plurality of
biological related materials disposed on a surface of said
substrate from said surface of said biochip using time of
flight secondary ion mass spectrometry, including at least:~~

~~—— a means for irradiating pulsed primary ion beam on said
surface of said biochip in a discontinuous pattern, said
surface of said biochip having said biological related
material disposed thereon, and said primary ion beam having
a spot size of smaller area than an area to be measured on
said surface of said biochip;~~

~~—— a means for conducting mass analysis of secondary ions
via time of flight, said secondary ion being generated by
irradiating said pulsed primary ion beam; and~~

~~—— a means for reconstructing analyzed results obtained by
conducting said mass analysis to form a two dimensional
information on the basis of said pattern of said irradiating~~

~~pulsed primary ion beam.~~

ABSTRACT OF THE DISCLOSURE

A measurement method is provided, which enables to obtain a two-dimensional image with better quantitative-ability by suppressing the influence of the charge~~ing~~-up, 5 when the two-dimensional secondary ion image is obtained for a biological-related material fixed on a substrate having a high resistivity by utilizing a TOF-SIMS method in a certainly wide area. ~~Two~~A two-dimensional image having considerably high positioning resolution-ability can be 10 obtained by the procedure, in which the pulsed primary ion beam is irradiated at a spot, and the pulse-wise spot-applications of the primary ion beam and the simultaneous detection of the secondary ion generated from the irradiated primary ion beam ~~are-proceeded~~ along with a discontinuous 15 scanning pattern, and eventually the results of these secondary ion measurements ~~results-is-are~~ are reconstructed into a two-dimensional image in line with the aforementioned discontinuous scanning pattern.